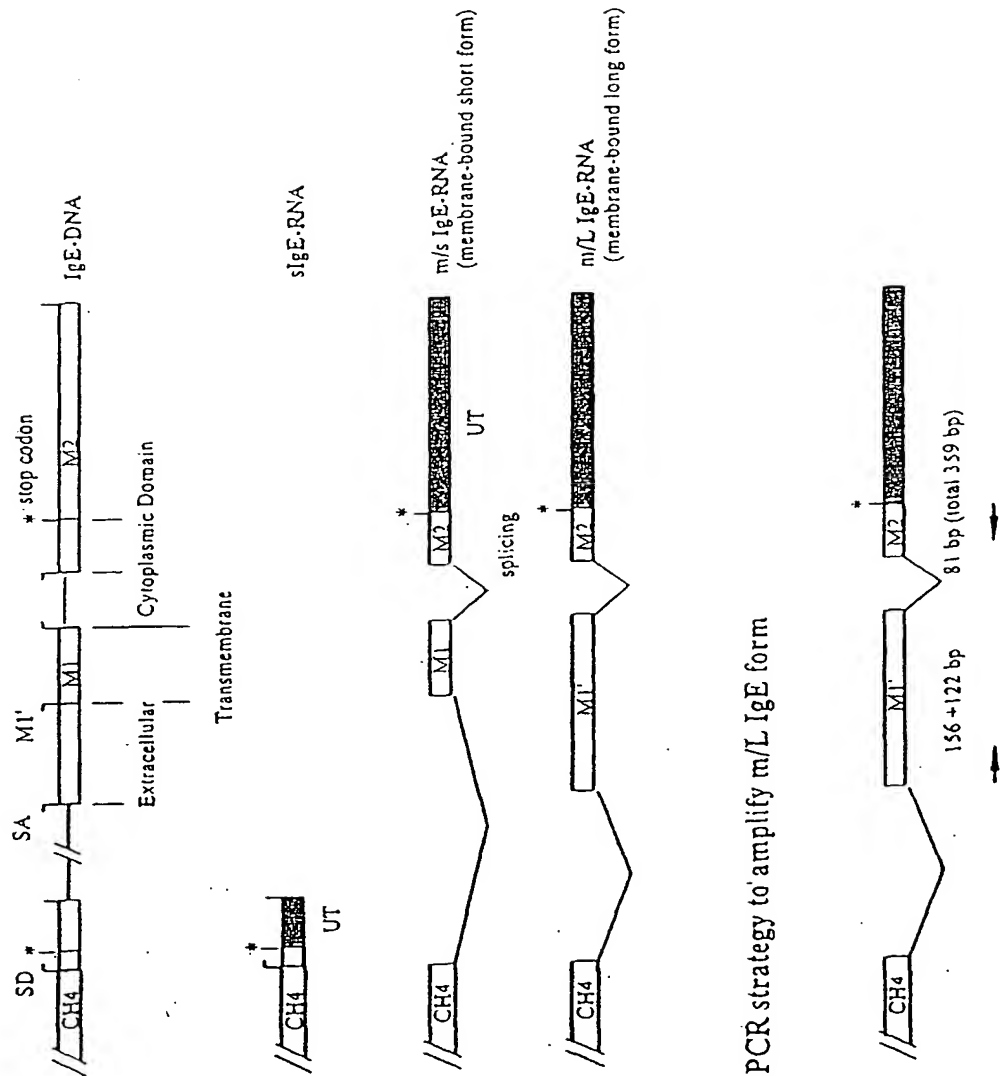


Construction of membrane-bound IgE

I: Cloning of human membrane-bound IgE gene by RT-PCR

A. Schematic representation of the secretory and two membrane human IgE H chain isoforms



B. RT-PCR strategy to amplify m/L IgE form

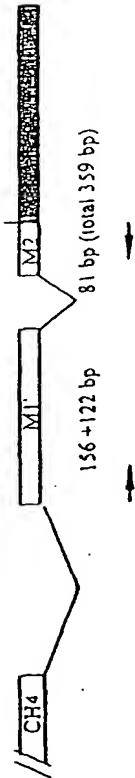
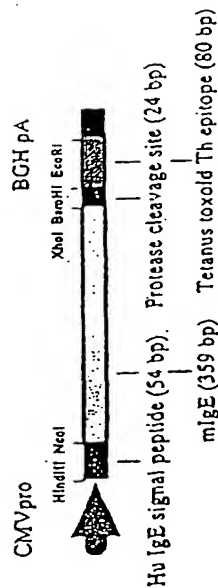


Fig.1

Construction of membrane-bound IgE

II. Construction of a vector insert for expression of a mIgE fused to TT

A. Human mIgE - Tetanus toxoid fusion protein expression cassette



B. PCR amplification with specific primer set

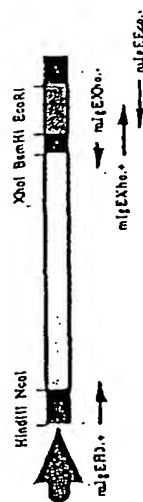


Fig.2

In Vitro Expression of mIgE Construct I.

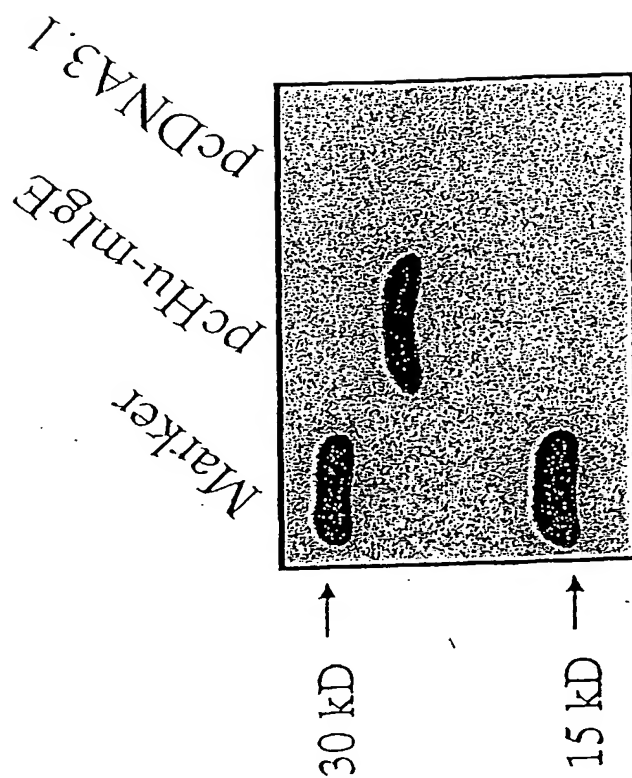


Fig.3

In Vitro Expression of mIgE Construct II.

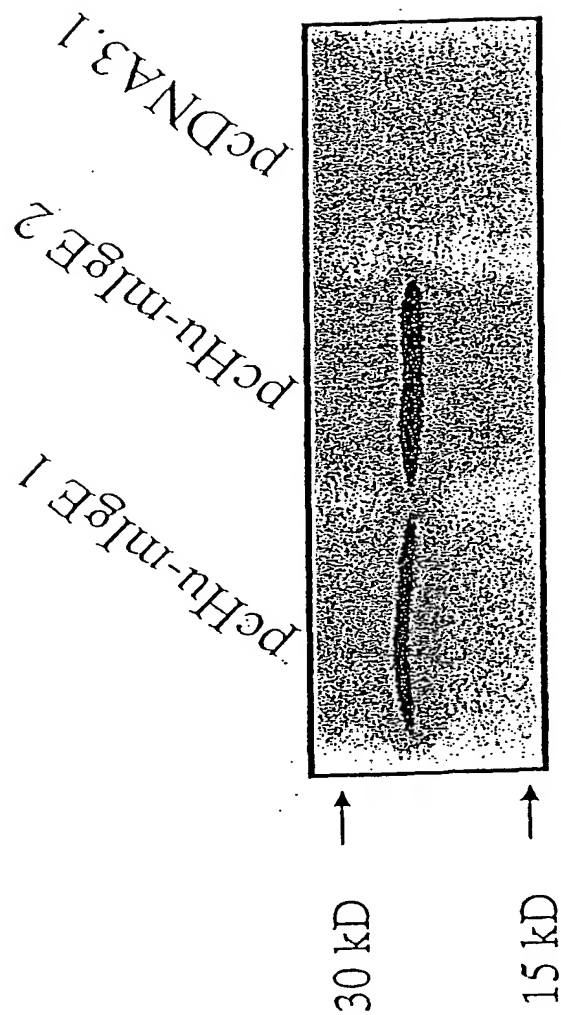


Fig.4

10/518701

5/8

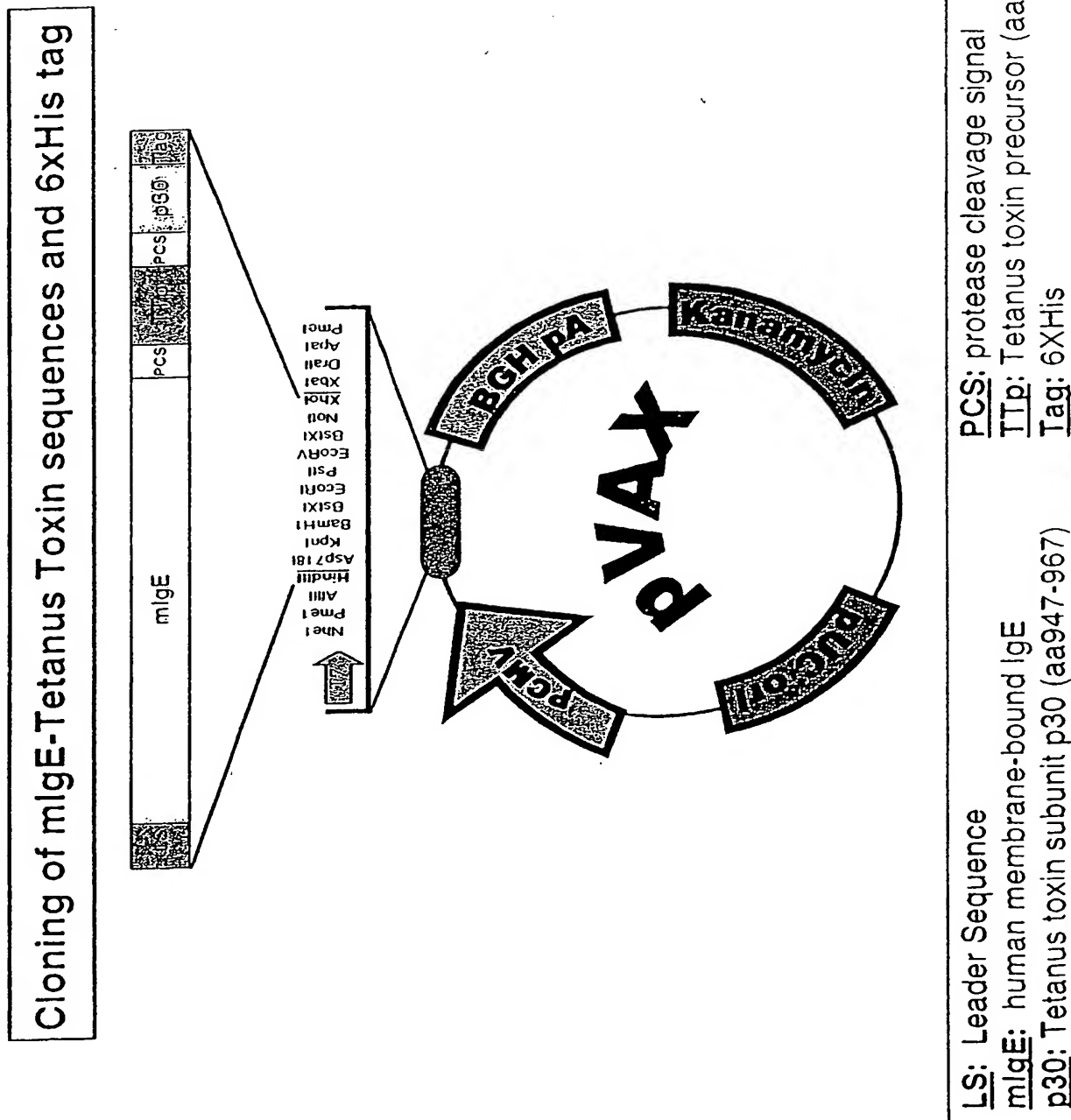


Fig.5

6/8

10/518701

mlgE-TT construct: Nucleotide and amino acid sequences

(SEQ ID NOS: 5 & 6)

atggactggacctggatcctcttcttgggtggcagcagccacgcgagtcactcccatggg

M D W T W I L F L V A A A T R V H S | H G

Leader sequence

ctggctggcggctccgcgcagtcaccagagggcccgga taggggtgctctgccactccgga

L A G G S A Q S Q R A P D R V L C H S G

mlgE

cagcagcagggactgccgagagcagcaggaggctctgtccccacccccgctgccactgt

Q Q Q G L P R A A G G S V P H P R C H C

mlgE

ggagccgggagggctgactggccaggtccccagagctggacgtgtgcgtggaggaggcc

G A G R A D W P G P P E L D V C V E E A

mlgE

gagggcgaggcgccgtggacgtggaccggcctctgcactcttcgcgcactcttctctgtc

E G E A P W T W T G L C I F A A L F L L

mlgE

agcgtgagctacagcgccgcctcacgctcctcatgggtgcagcgggttcctctcagccacg

S V S Y S A A L T L L M V Q R F L S A T

mlgE

cggcaggggagggccccagacctccctcgactacaccaacgtcctccagccccacgccaga

R Q G R P Q T S L D Y T N V L Q P H A | R

mlgE

gaaaaaagagctgttggttggttacgatccaaattatttaaggactgattctgataaagat

E K R A V V G | Y D P N Y L R T D S D K D

Protease cleavage signal

TTp

agatttttacaaaccatgggtaaaactgtttaacagaattaagagagaaaaaagagctgtt

R F L Q T M V K L F N R I K | R E K R A V

TTp

Protease cleavage

gttggttttaataattttaccggttagcttttggttgagggttcctaaagtatctgctagt

V G | F N N F T V S F W L R V P K V S A S

signal

p30TT

catttagaacatcatcatcatcatcattag

H L E | H H H H H H | -

Flag

Fig.6

10/518701

7/8

Synthetic peptides used as antigens for in vitro assays

Name	Amino acid sequence	Human mIgE Region
IgEEx#1	(SEQ ID NO: 7) SAQSQRAPDRVLCHSGQQQLP	extracellular (22 aa)
IgEEx#2	(SEQ ID NO: 8) AGGSVPHPRCHCGAGRADWPGP	extracellular (22 aa)
Migis	(SEQ ID NO: 9) ELDVCEEEAEGEAPW	extracellular (15 aa)
CTL2	(SEQ ID NO: 10) EAPWTWTGL	extracellular-transmembrane (9 aa)
CTL1	(SEQ ID NO: 11) TGLCIFAALF	transmembrane (9 aa)
IgECyt	(SEQ ID NO: 12) VQRFLSATRQGRPQTSLDYTNVLQPHA	intracellular (27 aa)
TTh (27 aa)	(SEQ ID NO: 13) YDPNYLRTDSDKDRFLQTMVKLFNRIK	

Fig. 7

10/518701

8/8

IFN- γ production determined in splenocytes from immunized mice
(7 days after 3rd immunization) after stimulation with IgE peptide antigen

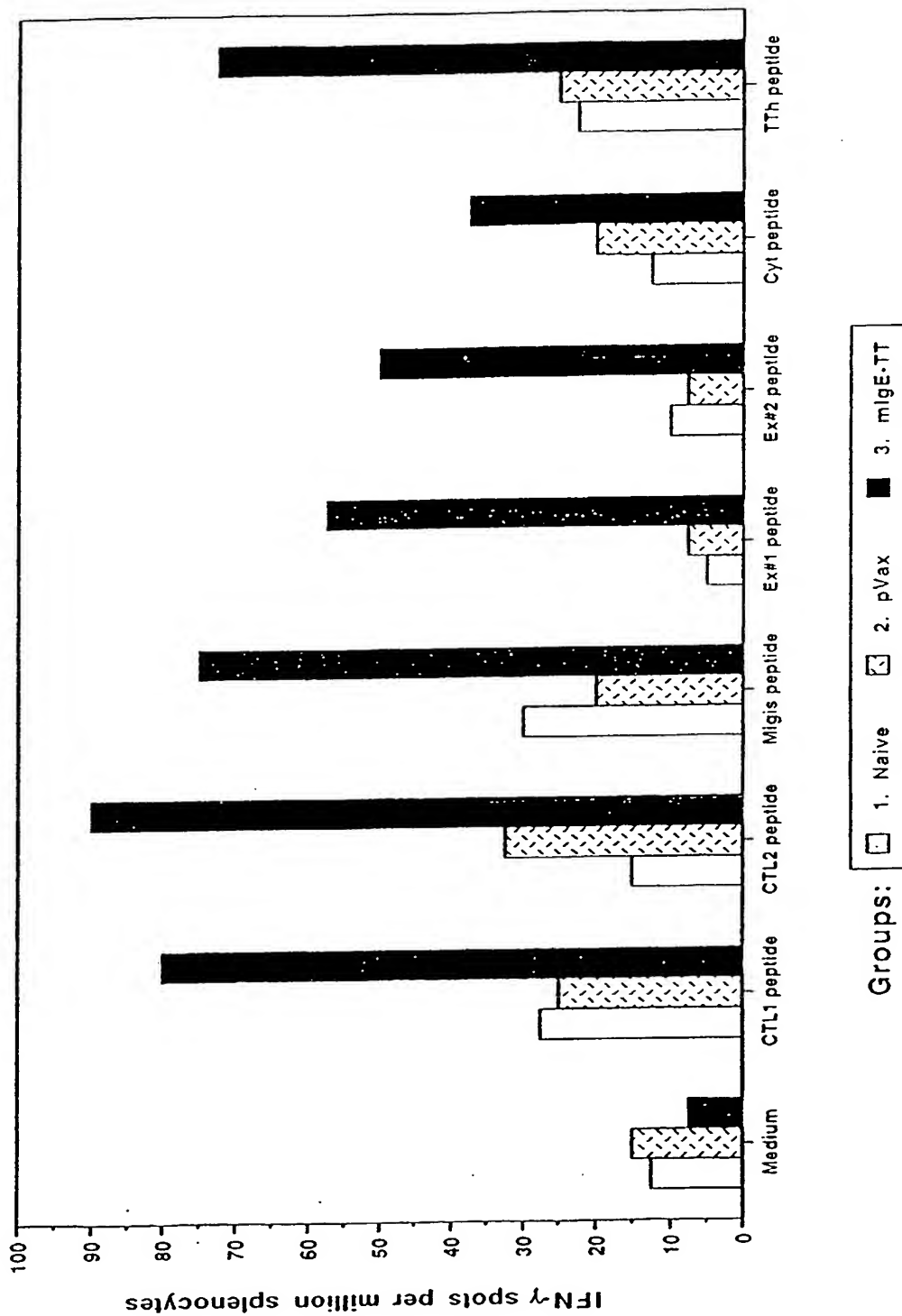


Fig.8